Attorney Docket: 10044

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Bootland et al.		Art Unit: 1645
Serial No.: 10/733,031		
Filed: December 11, 2003		Examiner: T. Phuong Bui
For:IMMUNIZATION OF FISH WITH PLANT-EXPRESSED RECOMBINANT PROTEINS		

DECLARATION OF DR.LINDA M. BOOTLAND UNDER 37 C.F.R. §1.132

Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Linda Bootland, hereby declare and state:

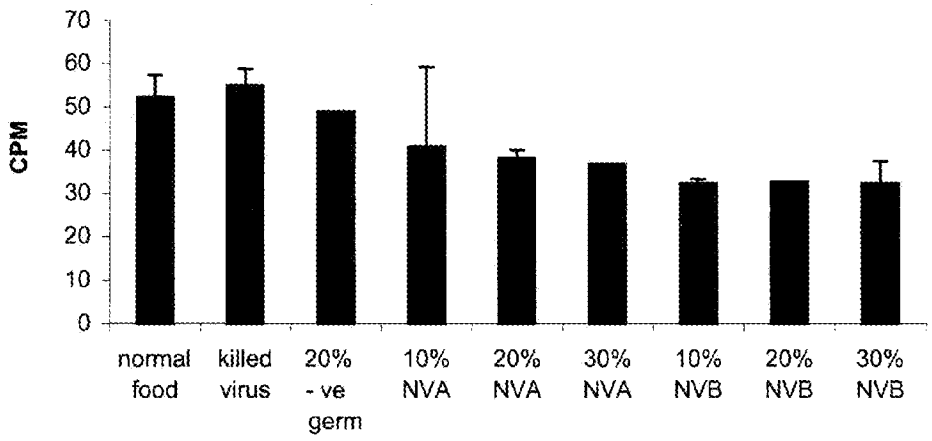
1. I am a Senior Research Scientist with Novartis Animal Health Canada Inc., the assignee of the above-named application and a named inventor. I am also an adjunct professor of the Department of Pathology and Microbiology at the University of Prince Edward Island. Previously I was a Principal Investigator with DiagXotics, Inc. I have a Ph.D. in microbiology from the University of Guelph, Ontario, Canada and received my Bachelor of Science degree, with honors, in marine biology and fisheries science from the University of Guelph. I have had 21 years working in the fisheries and biological sciences.
2. I have conducted experiments in which plant tissue having a nucleotide sequence expressing a fish antigen was fed to fish and a protective response obtained. In my experience in vaccinating fish against a pathogen, it was not predictable whether the fish when presented with a plant-produced antigen to a pathogen would be protected when then challenged with the pathogen. There are several reasons for this. One is that the protective response and digestive system of a fish is different from mammals since fish are cold

blooded and the immune system is less structurally defined than mammals. It is possible that a fish protective response is not antibody mediated but cell mediated and when a fish is exposed to an antigen, an antibody response may be obtained, and yet the fish is not protected against the pathogen. See for example Frost et al. "Analysis of the antibody response in Atlantic salmon against recombinant VP2 of infectious pancreatic necrosis virus (IPNV)" *Fish & Shellfish Immunology* (1998) 8:447-456, reporting antibody production does not necessarily provide protection (page 453). Further, the protein can be destroyed in the intestinal tract. Whether a plant-produced antigen will form and fold properly to elicit protection is also unknown. These problems are discussed, for example, in Barratt et al. WO 92/06599 (which employed a water-in-oil emulsion of an antigen to attempt to solve the issue); and in Companjen et al. "Development of a cost-effective oral vaccination method against viral disease in fish" Midtlyng PF (ed): *Progress in Fish Vaccinology. Dev Biol Basel*, Karger, 2005, vol. 21 pp. 143 – 150 (see especially pages 143-144) (copies appended). In Campanjen et al., the authors report in their study expression of LtB and influenza-parvo fusions in potatoes followed by anal intubation in fish resulting in uptake by the fish gut. But, as the authors also noted, "whether fish are indeed protected upon oral vaccination by plant-produced vaccines still has to be determined."

3. In conducted experiments, a protective response to IPNV was provided following oral administration of fish of a fish antigen by feeding plant material producing recombinant VP2 and VP3 antigens. To my knowledge this is the first time that a protective response to a pathogen has been obtained by feeding a fish plant material expressing a recombinant antigen to the pathogen.
4. I was provided with corn germ produced according to the patent application of the present invention as described in the specification at Example 3, starting at page 21, line 1 continuing to page 24 line 15. NVA represents the corn germ containing VP2 and VP3 expressing nucleotide sequences linked to the barley

alpha amylase signal sequence, allowing for the possibility of glycosylation; NVB is the same, but does not contain the signal sequence.

5. I conducted three separate non-optimized studies using the oral delivery of the transgenic germ containing NVA and NVB and was able to see protection in two of the three studies. Overall, an improved relative survival rate of up to 38% was observed. In one study no protection was provided. In the second study protection of up to about 38% relative survival rate was observed, with the exception of using 20% NVB, in which case mortality was higher than the negative control in one of the two reps, and thus appears to be an anomaly. A third study showed an improved relative survival rate of up to 38%, as shown in the figure below (CPM refers to cumulative percent mortality). The data demonstrates to a fish biologist that oral immunization of fish with fish antigens produced in plants induces protection.



I further declare that all statements made herein are of my own knowledge and are true and that all statements made on information and belief are believed to be true; and further than all statements made herein were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under

Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issuing hereon.

Linda Bootland

Dr. Linda M. Bootland

Dated: Mar. 1/07